

THE DETERMINATION OF FILTERED NITRATE NITROGEN

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THE DETERMINATION OF FILTERED NITRATE NITROGEN

Nitrates are natural constituents of plants, being present in significant quantities in many vegetables and to a lesser extent in fruit. Potential sources of nitrate in the environment arise from the agricultural use of fertilizers, nitrogen fixation of micro-organisms and plants, decomposition of sewage wastes, leaching from soil and rocks etc. Nitrates are formed via the oxidization of nitrite by autotrophic nitrifying bacteria and represent the most highly oxidized form of nitrogen within the nitrogen cycle. Surface waters generally contain trace levels of nitrate ion while ground waters may contain significant concentrations due to soil leaching. Nitrate in water is present in the dissolved or ionic form and is potentially an alternate source of oxygen when dissolved oxygen has been depleted.

Nitrate is one of several nutrients responsible for acceleration of the natural eutrophication process in surface waters. Excessive inputs tend to promote abnormal aquatic plant growth which results in the deterioration of recreational facilities such as boating, swimming, water skiing etc. In drinking waters, excess amounts can contribute to a disease known as infant methemoglobinemia in which the oxygen carrying capacity of the blood is inhibited. The maximum acceptable level for domestic water supplies in Ontario is 10 mg/l nitrate nitrogen. Nitrate present in significant amount in water supplies used for farm animals is known to have deleterious effects.

Nitrate results are useful for interpreting the effectiveness of the waste stabilization process at sewage treatment plants. The abundance of nitrate in the treated water gives a good indication of how far the treatment has progressed. Biochemical reduction of nitrates to nitrogen gas rather than ammonia (denitrification) is undesirable since the effectiveness of the activated sludge process is reduced. It is beneficial to complete the nitrification process because the presence of ammonia in the receiving water exerts an oxygen demand as well as being toxic to fish.

1. Sample Handling and Preservation

Glass or plastic containers are acceptable. Ammonia is rapidly nitrified by bacterial action in some samples and in order to obtain a nitrate value representative of conditions at the time of sampling, samples should either be frozen on site or refrigerated and shipped rapidly to the laboratory for immediate analysis. Acid preservation must be avoided due to chemical conversion of one or more of the nutrient parameters.

2. Selection of Method

Filtered samples are analyzed using an AutoAnalyzer system wherein nitrate is reduced to nitrite and the latter's concentration is determined by the formation of an azo dye (see the Determination of Filtered Nitrite Nitrogen). In Method A, a granular cadmium column is used in the reduction step while Method B employs a hydrazine solution. The azo dyes formed also differ slightly in composition. The range of application of both methods is limited only by the dilution required to obtain a satisfactory response.

All four filtered nutrient analyses (reactive phosphates, nitrate, nitrite and ammonia) are performed simultaneously on the same filtered aliquot. This provides greater efficiency in sample handling and in productivity but introduces some constraints on the test procedure. In any manipulation of the sample prior to the completion of filtration, the effect on *all four* tests must be considered. In particular, care must be taken in the selection of preservatives, and in the prevention of contamination of filter papers and apparatus to avoid introducing undetected variations in the blanks or test results for any of the four tests.

FILTERED NITRATE NITROGEN
CADMIUM REDUCTION - DIAZOTIZATION METHOD A

SUMMARY	
Substance determined.	Nitrate ion, NO_3
Interpretation of results.	Since the analysis measures nitrate plus nitrite, a separate determination of nitrite is required in order to obtain the desired result. Ground water concentrations of nitrate nitrogen greater than 10 mg/l exceed the drinking water criteria.
Principle of method.	A filtered aliquot of sample is passed over granular metallic cadmium where nitrate is reduced to nitrite. After diazotization with sulphanilic acid and coupling the reaction product with 1-naphthylamine, buffering the solution to a pH of 3.7 produces a pink colour. The absorbance of the solution is measured at 520 nm and the concentration of nitrate plus nitrite nitrogen is determined by comparison with a similarly treated series of standards. The nitrate concentration is obtained by a difference calculation: $\text{NO}_3 \text{ nitrogen} = (\text{NO}_3 + \text{NO}_2 \text{ nitrogen}) - (\text{NO}_2 \text{ nitrogen})$.
Time required for analysis.	Approximately 200 samples may be analyzed during an 18 hour AutoAnalyzer work period.
Range of application.	Minimum reported value $\pm 0.01 \text{ mg N/l}$. The absorption curve obeys Beer's law over the normal operating range of 0.02 to 2.0 mg/l. Concentrations exceeding 2.0 mg/l are determined by dilution.
Standard deviation.	± 0.02 on an undiluted sample in the operating range of 0.02 to 2.0 mg/l.
Accuracy.	Within precision of test.
Limit of detection.	0.02 mg/l nitrogen.
Interferences and shortcomings.	Certain metallic ions may precipitate during the analysis (i). Strong oxidizing and reducing agents, greases and oils may alter

SUMMARY

the reduction process on the cadmium surface. Method requires separate nitrite nitrogen determination.

Minimum volume
of sample.

75 ml.

Preservation and
sample container.

Glass or plastic bottles are acceptable. Refrigeration and rapid shipment to the laboratory are necessary to maintain sample integrity. Acid preservation must be avoided.

Safety
considerations.

Crystalline α -naphthylamine reagent may contain up to 1% β -naphthylamine which is known to be carcinogenic. The solid reagent should be handled in such a manner as to prevent the powder from becoming airborne.

Heavy gloves and safety glasses are required when preparing cadmium filings.

FILTERED NITRATE NITROGEN
CADMIUM REDUCTION - DIAZOTIZATION METHOD A

1. Introduction

A filtered portion of the sample is presented to the AutoAnalyzer, where, as one of four concurrent tests, a proportioned aliquot is withdrawn into the nitrate channel either directly or through a dilution loop as required. EDTA reagent is mixed with the sample and then passed through a column of cadmium metal filings where nitrate ion is electrochemically reduced to nitrite ion. After passing through the cadmium column, the sample stream is segmented with air and colour reagent containing α -naphthylamine and sulphanilic acid are added. Nitrite ion reacts with sulphanilic acid to form a diazotization product which couples with the naphthylamine. Buffering the solution to a pH of approximately 3.7 produces a light red chromophore in proportion to the concentration of nitrite ion originally present in the solution and the nitrite that is produced by the reduction of nitrate. The absorbance of the solution is measured colorimetrically in a 5 cm flow cell at 520 nm. The concentration of nitrite plus nitrate in mg/l nitrogen is obtained by comparing sample peak heights on the recorder trace against those obtained from a similarly tested series of standards. The concentration of $\text{NO}_3\text{-N}$ in mg/l is obtained by subtracting the sum of $(\text{NO}_2 + \text{NO}_3) - \text{N}$ from the $\text{NO}_2 - \text{N}$ result; the latter is obtained in a separate analysis.

2. Interferences and Shortcomings

Strong oxidizing or reducing agents should be absent. Metal ions that precipitate under the test conditions must be removed.

Cupric ions may produce low results by catalyzing the decomposition of the diazonium salt.

Although the nitrite concentration of natural waters is usually quite low, a separate determination of nitrite must be completed to obtain an actual nitrate concentration. Poisoning of the cadmium column will alter the recovery of the reduction process. Within-run sensitivity checks are required to monitor the activity of the column.

A separate nitrite determination must be performed in order to obtain a unique nitrate determination.

3. Apparatus

- a) Filtration apparatus: see Figure 1(a), Determination of Filtered Ammonia.
- b) Filter paper, glass fiber, 4.25 cm Reeve Angel 934AH.
- c) Instrument.
 - i) sampler
 - ii) proportioning pump
 - iii) colorimeter equipped with 520 nm filters and a 5 cm flow cell.
 - iv) voltage regulator.
 - v) chart recorder.
- d) Pump tubing manifold and associated manifold glass ware as shown in Figure 1(b) for AAI or Figure 1(c) AAI systems.
- e) Culture tubes 19 X 150 mm.
- f) Culture tube racks of 40 tube capacity.
- g) Dilution tubes, 50 ml capacity.
- h) Nessler or dilution tubes, 100 ml capacity.
- i) Reagent bottles, pyrex, bulk storage 9 l
- j) Reagent reservoir bottles, pyrex, low actinic, 2 l, 150 ml.

4. Reagents

- a) Potassium Nitrate, KNO_3 , anhydrous, reagent grade powder.

POTASSIUM NITRATE IS A STRONG OXIDANT WHICH MAY INDUCE SPONTANEOUS COMBUSTION WHEN IN CONTACT WITH CERTAIN CARBONACEOUS MATERIALS.

- b) Disodium EDTA dihydrate, $[CH_2N(CH_2COOH)CH_2COONa]_2 \cdot 2H_2O$, reagent grade powder.

- c) Cadmium metal, Cd , reagent grade, stick form.

- d) Cadmium reduction column

Cadmium metal filings (no large than approximately 2 mm long by 1 mm in width) are obtained by filing a cadmium stick with a coarse rasp. Pack the chips loosely to a length of 6 mm in a $6\frac{1}{2}$ mm X 4 mm O.D. piece of glass tubing. Insert plugs of glass wool in each end of the column to prevent loss of filings and attach suitable connectors. The cadmium column is cleaned by drawing a saturated EDTA solution through it via a light vacuum.

GLOVES SHOULD BE WORN DURING THE RASPING PROCESS TO PREVENT CONTACT OF THE CADMIUM METAL AND SKIN.

Additional supporting solutions for the nitrate test are identical to the solutions required for the Determination of Filtered Nitrite Nitrogen Method A, see section 4h through 4n.

- e) EDTA Reagent

Dissolve 5.0 grams of disodium EDTA dihydrate in deionized distilled water and add 25 ml of stock Buffer A before diluting to 1 liter.

- f) Concentrated Stock Standard Nitrate Solution

Dissolve 13.00 g of anhydrous reagent grade potassium nitrate, KNO_3 , in deionized distilled water and dilute to 1 liter in a volumetric flask. Mix well and store in a tightly stoppered container under refrigeration. Concentration of $NO_3-N = 1800$ mg/l.

g) Dilute Stock Standard Nitrate Solution

Dilute a 20.0 aliquot of the concentrated stock standard nitrate solution to 1 liter in a volumetric flask. Concentration of $\text{NO}_3\text{-N} = 36 \text{ mg/l}$.

h) Working Stock Standard Nitrate Solution

i) High range.

Dilute a 10.0 ml aliquot of the concentrated stock standard nitrate solution to 1 liter in a volumetric flask. Concentration of $\text{NO}_3\text{-N} = 18 \text{ mg/l}$.

ii) Intermediate range.

Dilute a 50.0 ml aliquot of the dilute stock standard nitrate solution to 1 liter in a volumetric flask. Concentration of $\text{NO}_3\text{-N} = 1.8 \text{ mg/l}$.

iii) Low range.

Dilute a 20.0 ml aliquot of the dilute stock standard nitrate solution to 1 liter in a volumetric flask. Concentration of $\text{NO}_3\text{-N} = 0.720 \text{ mg/l}$.

j) Working Standard Solutions

Daily calibration standards are prepared by diluting the following aliquots to fifty (50) ml.

i) High Range Working Standards - use high range working Stock Standard Nitrate Solution, 18 mg N/l.

High range stock ml 50.0 40.0 30.0 20.0 10.0 5.0

Working Standards						
mg N/l	18.0	14.4	10.8	7.2	3.6	1.8

ii) Intermediate Range Working Standards - use Intermediate Range Working Stock, 1.8 mg N/l.

Intermediate Stock	50.0	40.0	30.0	20.0	10.0	5.0
ml						

Working Standards	1.80	1.44	1.08	.72	.36	.18
mg N/l						

- iii) Low Range Working Standards - use Low Range
Working Stock, 0.72 mg N/l.

Low Range Stock	50.0	40.0	30.0	20.0	10.0	5.0
ml						

Working Standards	0.720	0.576	0.432	0.288	0.144	0.072
mg N/l						

Note: The nitrate method measures $\text{NO}_2 + \text{NO}_3$ and not strictly NO_3 . The standards for NO_2 and NO_3 on similar ranges are combined to give the final expected value. For example, on the intermediate range the high standard 2.0 mg/l is composed of 1.8 mg $\text{NO}_3\text{-N/l}$ and 0.2 mg $\text{NO}_2\text{-N/l}$.

k) Quality Control Samples

Prepare Quality Control A and B solutions, QC-A and QC-B respectively, that will provide test solution for at least 20 days of analysis. The concentration of QC-A and -B should be chosen such that they fall within the normal concentration range of sample being routinely analyzed. These quality control checks are used to detect systematic errors such as blank or calibration changes from day to day and must be included in each run of standards and samples on a day to day basis. Prepare a new QC-A and QC-B and monitor their concentration for at least 3 days prior to adopting them.

l) Daily Sensitivity Checks

i) AAI Systems.

In order that sensitivity changes within a run can be monitored, prepare solutions that will provide 100% (high) and 10% (low) of full scale response.

ii) AAI Systems

In order that sensitivity changes within a run can be monitored, prepare solutions that will provide 80% (high) and 10% (low) of full scale response.

5. Procedure

The procedure of collecting, grouping, and filtering of samples, loading the AutoAnalyzer sampler, determining the calibration curve and interpretation of individual peak heights is identical to that described in detail in Filtered Ammonia. See Procedure: Determination of Filtered Ammonia.

6. Calculation and Method of Reporting

Multiply the reading by the dilution factor

$$\frac{\text{dilution volume}}{\text{aliquot volume}}$$

and record the result in the answer blank opposite each sample number. Report the results according to the following schedule.

Low Range: A maximum of *three* figures are shown when reporting to the *second* decimal place with a minimum reporting value of less than 0.005 mg N/l.

High range: A maximum of *two* significant figures are shown when reporting to *one* decimal place with a maximum reporting value of less than 0.01 mg N/l.

7. Precision and Accuracy

Standard Deviation in mg/l	Duplicates	Concentration in mg/l NO ₃ as N
± 0.020	within-run	0.40
± 0.055	between-run	1.63
± 0.148	between-run	8.04

8. Bibliography

- i) Standard Methods for the Examination of Water and
Wastewater, 13th ed., APHA; Washington, D.C; 1971.*

FILTERED NITRATE NITROGEN
HYDRAZINE REDUCTION - DIAZOTIZATION METHOD B

SUMMARY

Substance determined.	Nitrate ion, NO_3 .
Interpretation of results.	As the analysis measures nitrate plus nitrite, a separate determination of nitrite is required in order to obtain the desired result. Ground water concentrations of nitrate nitrogen greater than 10 mg/l exceed the drinking water criteria.
Principle of method.	The nitrate content of the sample is reduced to nitrite by heating a filtered aliquot with hydrazine in alkaline media; this reaction is catalyzed by cupric ion. Subsequently an azo dye is formed in acid media by diazotizing sulphanilamide with nitrite and coupling the product with N(1-naphthyl) ethylenediamine dihydrochloride. The absorbance of the pink azo dye is measured at 520 nm and the concentration of $\text{NO}_3\text{-N}$ is determined by comparison with a similarly treated series of standards.
Time required for analysis.	Approximately 20 analyses can be performed in an hour and over 200 per day.
Range of application.	Minimum reported value 0.02 mg N/l . Concentrations exceeding 1 mg N/l are determined by in-line or manual dilution.
Standard deviation.	0.01 mg N/l .
Accuracy.	Within precision of the test.
Limit of detection.	0.02 mg N/l .
Interferences and shortcomings.	The pH of the sample must lie between 6 and 9. Cupric and ferrous concentrations should be less than 4 and 2 mg/l respectively. Oxidizing agents can prevent reduction of nitrate and reducing agents can destroy the nitrate content of the sample.

SUMMARY

Minimum volume of sample. 75 ml

Preservation and sample container. Glass, polyethylene or styrene bottles are acceptable. Refrigeration and rapid shipment of samples is essential. Acid preservation must be avoided.

Safety considerations. Normal safety precautions for handling strong bases and acids are required. As all naphthyl type compounds are considered potential carcinogens until proven otherwise, care should be taken in handling the powder, N(1-naphthyl) ethylenediamine dihydrochloride.

FILTERED NITRATE NITROGEN
HYDRAZINE REDUCTION - DIAZOTIZATION METHOD B

1. Introduction

An aliquot of filtered sample is pumped into the nitrate channel directly or through a dilution loop. This sample stream is segmented with air and mixed with fixed portions of sodium hydroxide (to adjust the pH to 12.2), hydrazine (to reduce the nitrate to nitrite), copper sulfate (to catalyze the latter reaction); this combined stream is then heated to 37.5°C to complete this reduction phase of this analysis. The nitrite solution issuing from the heater is mixed with a color reagent which contains hydrochloric acid (to adjust the pH to 1.0 ± 0.1), sulphanilamide (for the diazotization reaction with nitrite) and N(1-naphthyl) ethylenediamine dihydrochloride, the coupling agent to produce the pink azo dye. The absorbance of the solution is measured with a colorimeter at 520 nm using a 5 cm flow cell and recorded as a peak on a chart recorder. The result in mg N/l is obtained by comparison with similarly treated nitrate standards.

2. Interferences and Shortcomings

If an excess of reducing agents, e.g. sulphide or ferrous ions are present, nitrate will be reduced beyond the nitrite form; if an excess of oxidizing agents, e.g. dichromate are present, the hydrazine concentration will be insufficient to convert the nitrate to nitrite. The pH of the sample must be in the range 6 to 9 as the system cannot be buffered; if the sample pH is 2 to 6, the answer will be 5% low.

3. Apparatus

- a) Standard Technicon AAI System plus one non-adjustable temperature (37.5°C) cartridge, see Figure 1(d) and Figure 1(e) dilution loop used for sewage manifold.
- b) Volumetric flasks.
- c) Volumetric pipettes.
- d) Filtration units.
- e) Reeve-Angel glass fiber filter papers - 4.25 cm diameter.
- f) Dilution tubes.

4. Reagents

- a) Potassium Nitrate, KNO_3 , reagent grade crystals.
- b) Sodium Nitrite, $NaNO_2$, reagent grade crystals.
- c) Sodium Hydroxide, $NaOH$; reagent grade pellets.
- d) Hydrazine Sulphate, $N_2H_4 \cdot H_2SO_4$; reagent grade crystals.
- e) Copper Sulphate; $CuSO_4 \cdot 5H_2O$; reagent grade powder.
- f) Hydrochloric acid; HCl ; concentrated reagent grade.
- g) Sulphanilamide, $H_2N-C_6H_4SO_2NH_2$; reagent grade powder.
- h) N(1-Naphthyl) Ethylenediamine Dihydrochloride
 $C_{10}H_7HNCH_2CH_2NH_2 \cdot 2HCl$ (Marshall's Reagent)
reagent grade powder.
- i) Concentrated Stock Sodium Hydroxide

On a top-loading balance, weigh out 728 g of $NaOH$. Place approximately 600 ml of deionized distilled water in a 2 liter beaker and with continuous stirring cautiously add the $NaOH$ to the beaker. When the solution is cool, dilute to 2 liters with deionized distilled water.

- j) Working Sodium Hydroxide Solution

Pipet 25 ml of stock $NaOH$ into a 1 liter volumetric flask and make up to the mark with deionized distilled water.

EYE PROTECTION MUST BE WORN DURING THIS PROCEDURE. THIS SOLUTION IS 30% DRY WEIGHT $NaOH$ AND MAY CAUSE SEVERE BURNS TO UNPROTECTED AREAS.

- k) Stock Hydrazine Sulphate

Dissolve 24.17 g of hydrazine sulphate in 900 ml deionized distilled water and dilute to 1.0 liter. It may be necessary to apply heat in order to dissolve the hydrazine. This solution is stable for one month.

CAUTION - THIS REAGENT IS TOXIC IF INGESTED.

1) Working Hydrazine Solution

Pipet 50 ml of stock hydrazine and dilute to 1.0 liter in a volumetric flask. Prepare fresh daily as the solution deteriorates and loses sensitivity after 24 hours.

m) Stock Copper Sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$)

Dissolve 1.125 g of copper sulphate in deionized distilled water and dilute to 1.0 liter.

n) Working Copper Sulphate Solution

Dilute 10 ml of stock solution to 1.0 liter with deionized distilled water. The above solution is the minimum copper sulphate concentration required to catalyze the reduction reaction. If an excess is added, it will very slowly precipitate and re-dissolve cyclically; this causes loss of stability as shown by variation of the in-run standards.

o) Color Reagent

To approximately 500 ml of deionized distilled water, add 55 ml of concentrated hydrochloric acid. Dissolve 22.5 g of sulphanilamide and 1.123 g of N(1-naphthyl) ethylenediamine dihydrochloride in the acid solution and dilute to 1.0 liter. Filter if necessary.

THIS PROCEDURE MUST BE DONE IN A FUME HOOD WITH ADEQUATE SAFETY PRECAUTIONS TAKEN TO PROTECT EYES AND OTHER EXPOSED AREAS AGAINST ACID SPILLS.

p) Concentrated Stock Standard Nitrate Solution

Dissolve 13.0 g of anhydrous reagent grade potassium nitrate, KNO_3 , in deionized distilled water and dilute to 1 liter in a volumetric flask. Mix well and store in a tightly stoppered container under refrigeration. Concentration of $\text{NO}_3\text{-N}$: 1800 mg/l.

q) Working Stock Standard Nitrate Solution

Dilute 10.0 ml of the concentrated stock standard nitrate solution to 1 liter in a volumetric flask. Concentration of $\text{NO}_3\text{-N}$: 18 mg/l.

r) Working Standard Solutions

Daily calibration standards are prepared by diluting the following aliquots to fifty (50) ml.

Working Standards - Use Working Stock Standard Nitrate Solution, 18 mg/l N.

Working Stock, ml 50.0 40.0 30.0 20.0 10.0 5.0

Working Standards, 18.0 14.4 10.8 7.2 3.6 1.8
mg/l N

s) Reduction Step Check Solutions

Prepare separate solutions of NO_3 and NO_2 , 10 mg/l N each, to give a 50% full scale response on the NO_3 channel. These solutions are analyzed daily to monitor the efficiency of the reduction step in the test procedure.

t) Quality Control Samples

Prepare Quality Control A and B solutions, QC-A and QC-B respectively, that will provide test solution for at least 20 days of analysis. The concentration of QC-A and -B should be chosen such that they fall within the normal concentration range of samples being routinely analyzed. These quality control checks are used to detect systematic errors such as blank or calibration changes from day to day and must be included in each run of standards and samples on a day to day basis. Prepare a new QC-A and QC-B and monitor their concentration for at least 3 days prior to adopting them.

u) Daily Sensitivity Checks

AAII Systems

In order that sensitivity changes within a run can be monitored, prepare solutions that will provide 80% (high) and 10% (low) of full scale response.

5. Procedure

The basic procedure is similar to that described in the Filtered Ammonia test, with the exception that the reduction step check solutions must be included in the initial calibration and every 60 samples thereafter. These 50% full scale nitrate and nitrite standards must agree within 4% of the expected value. If the nitrate value is too low, one or more of the following may be true:

- a) the hydrazine solution has deteriorated.
- b) the copper sulphate concentration is insufficient.
- c) the pH leaving the heating bath is not 12.2 ± 0.1 .
- d) the pH at the flow cell is not 1.0 ± 0.1 .
- e) the manifold is not pumping the specified volumes due to a defective or aged tube.
- f) the temperature of the heating bath is not 37.5°C .

6. Calculation and Method of Reporting

If necessary multiply the reading by the dilution factor (diluted volume/aliquot volume) and subtract the nitrite nitrogen concentration for the same sample. Report the result as mg/l nitrate nitrogen according to the following schedule:

Working Range - a maximum of *two* significant figures are shown with a minimum reporting value of less than 0.2 mg N/l.

7. Precision and Accuracy

Between and within run standard deviations of ± 0.009 mg $\text{NO}_3\text{-N/l}$ have been determined in the range 0.02 - 1.00 mg $\text{NO}_3\text{-N/l}$.

8. Bibliography

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- iii) Jacobs, M.B., and Hochheiser, S., *Anal. Chem.* 30,
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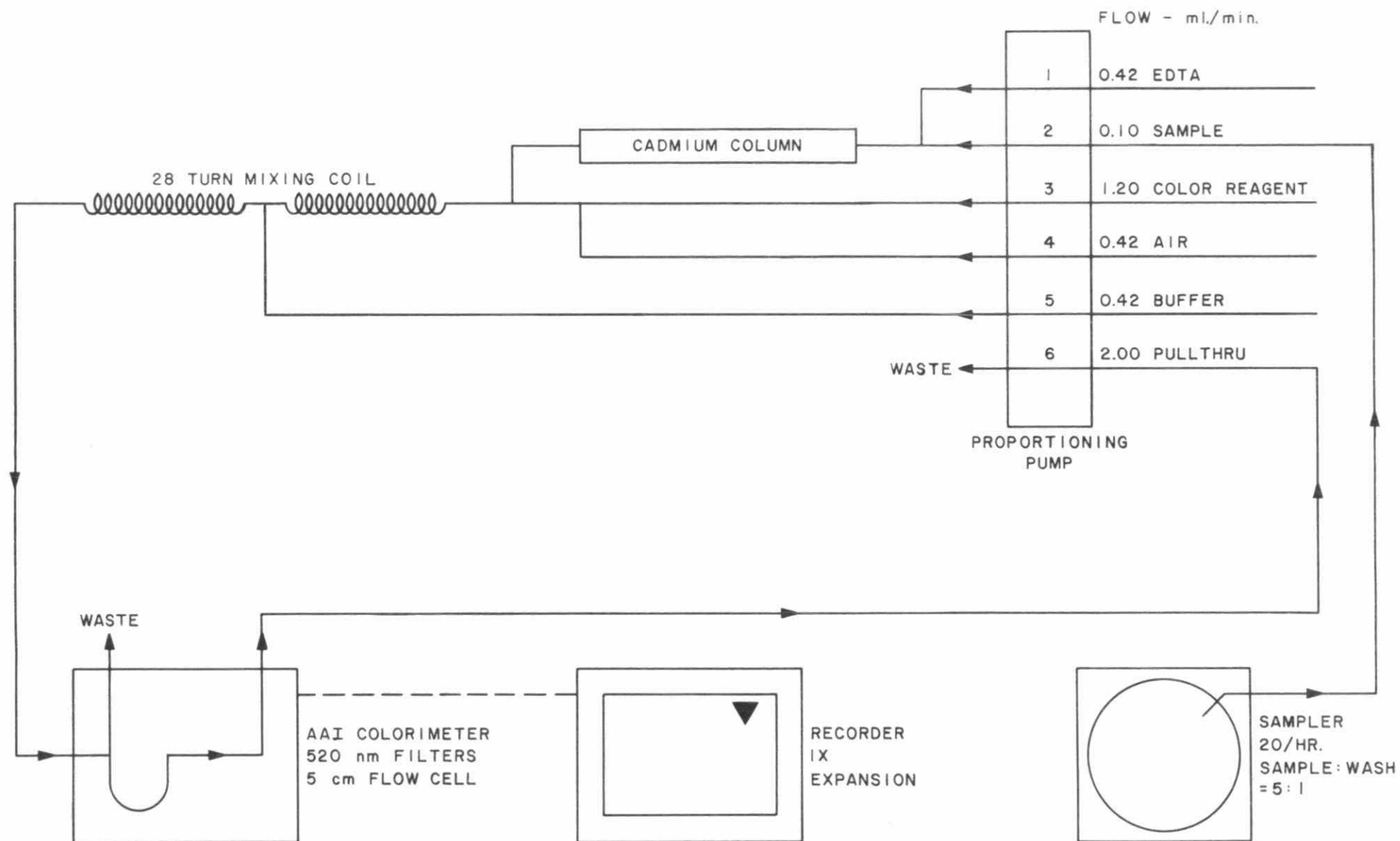


FIGURE 1(b) - AUTOANALYSER AAI SYSTEM FOR FILTERED NITRATE NITROGEN (HIGH RANGE) METHOD A

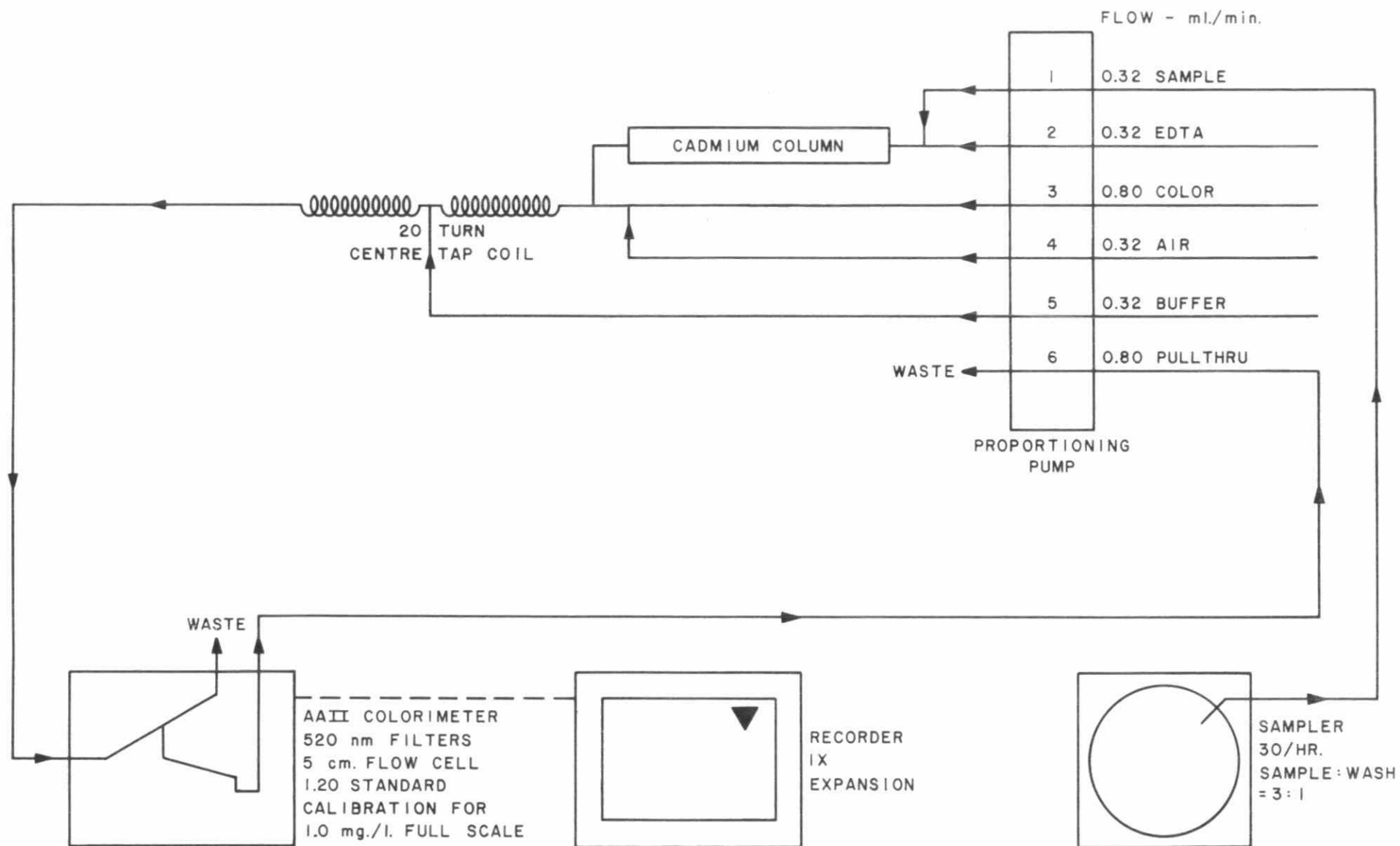


FIGURE 1(c) - AUTOANALYSER AAI SYSTEM FOR FILTERED NITRATE NITROGEN (LOW RANGE) METHOD A

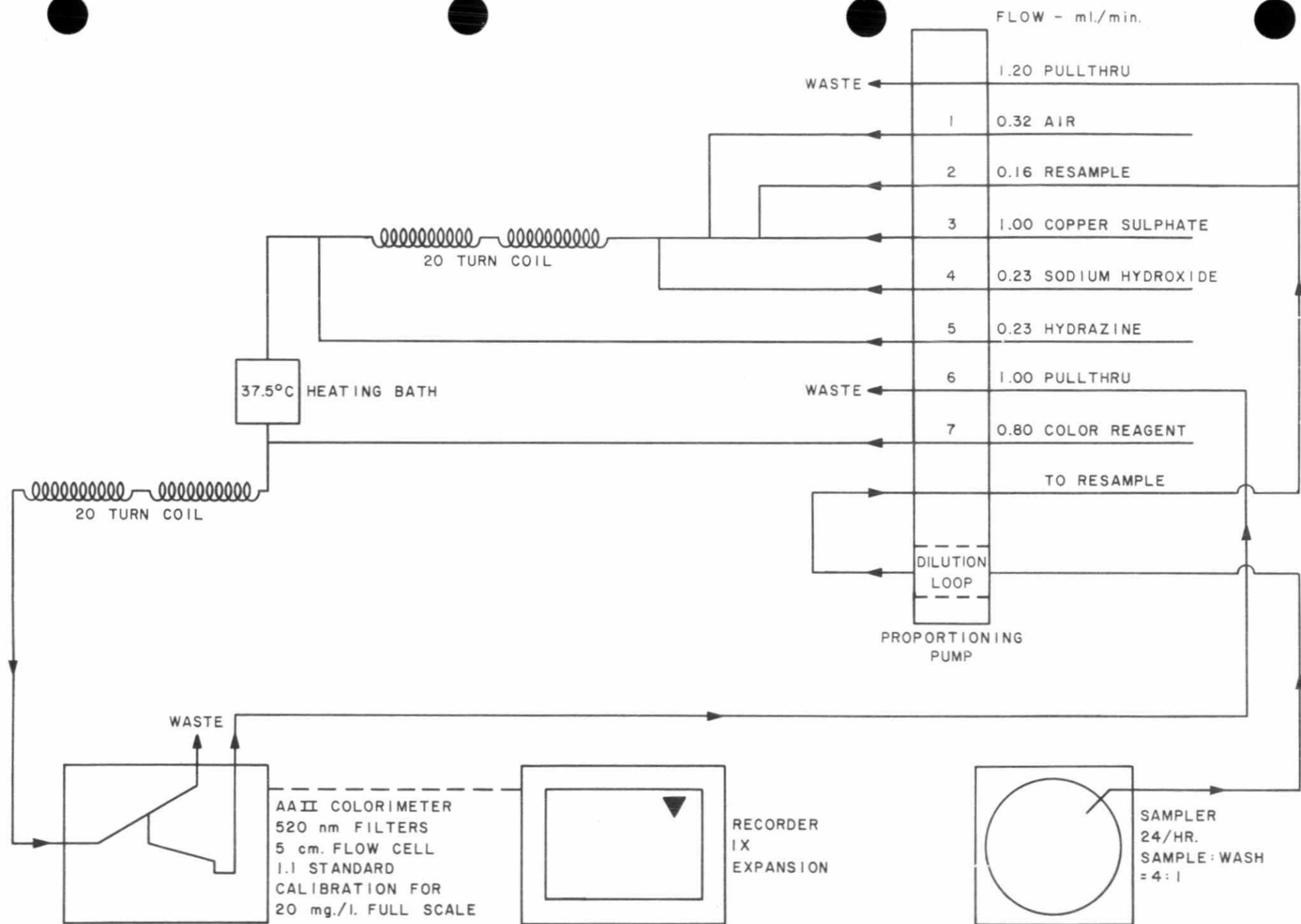


FIGURE 1 (d) - AUTOANALYSER AAII SYSTEM FOR FILTERED NITRATE NITROGEN METHOD B

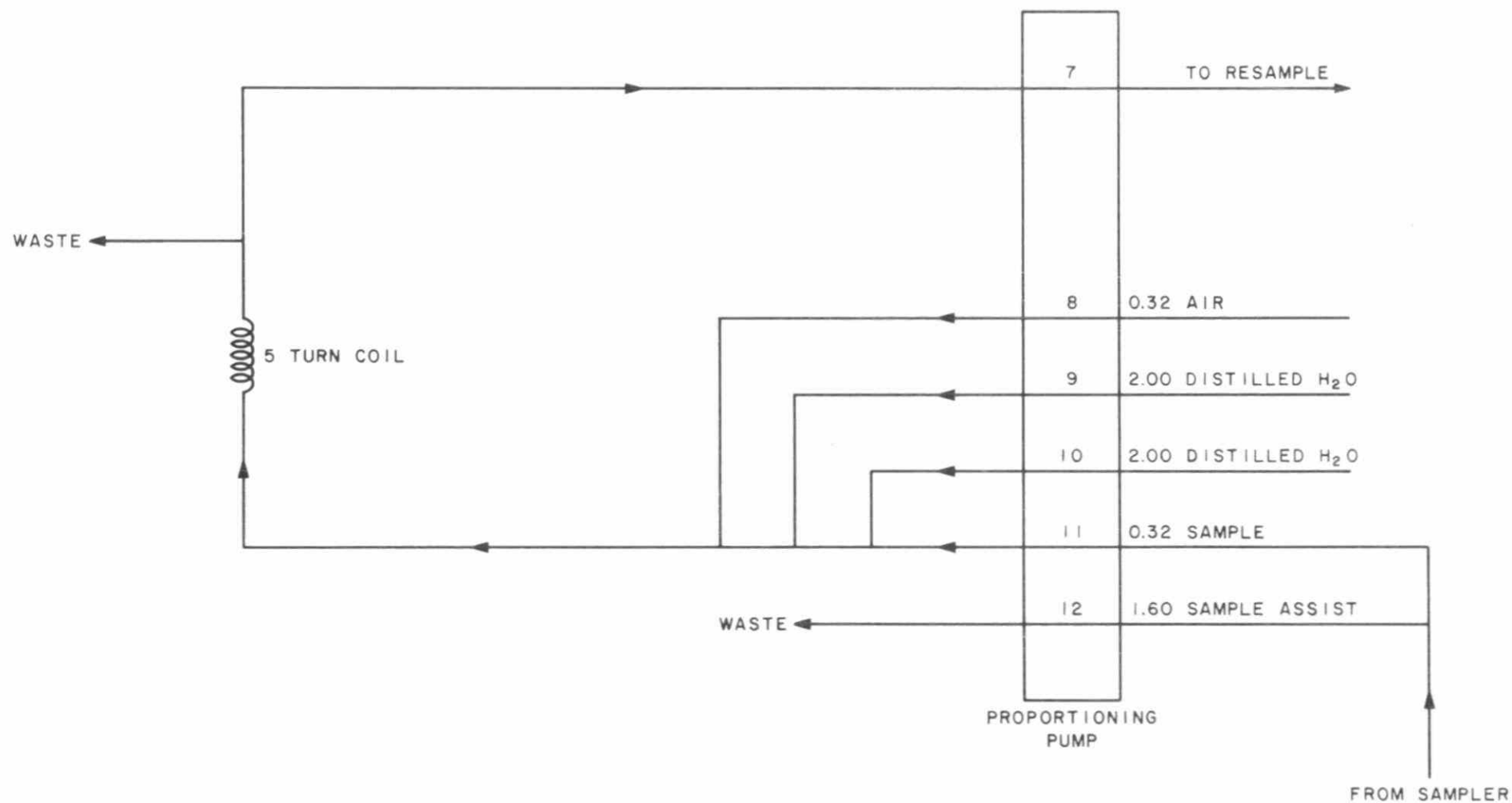


FIGURE 1(e) - DILUTION LOOP FOR SEWAGE MANIFOLD FILTERED NITRATE NITROGEN METHOD B



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